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Optimisation Of Sono Assisted Pretreatment Of SwitchGrass (*panicum virgatum l.*) For The Production of Reducing Sugars Using Response Surface Methodology (RSM)

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***Abstract-** The present work reports ultrasonic pre-treatment of Switchgrass (Panicum virgatum L.). The effects of different alkalis as the liquid medium were tested for the pre-treatment of switch grass and NH_4OH was found to give the maximum reducing sugars. Response surface methodology (RSM) was used to investigate the effect of process variables on the reducing sugars yield. Four independent variables i.e Alkali concentration (wt%), Sonication time (min), Solids Ratio (g/10ml of liquid medium) and Amplitude (%).The desirable pretreatment conditions found were Ammonia concentration 2.5 wt%, time of pretreatment 5.5min, solids ratio 0.15g/10mL of liquid medium, % amplitude 55%. Experimental runs under the aforementioned conditions resulted in a reducing sugar yield of 2.679g/l = 66.975 mg of reducing sugars / gram of biomass.*
Keywords: switchgrass, ultrasound, reducing sugars

I. INTRODUCTION

The world is in immediate need of new sources as liquid transportation fuels to address vital strategic, economic, and environmental problems. Furthermore, there is growing evidence that global conventional oil use is nearing the point where half of the accessible reserves have been depleted, pointing toward the real possibility that production will not be able to keep up with demand in the near future [1].This situation is compounded by the tremendous growth in oil demand by China, India and other developing countries. The term bio-fuel is referred to as liquid or gaseous fuels for the transport sector that are predominantly produced from biomass [2]. One of the major drivers for worldwide biofuels development is the concern about global climate change that is primarily caused by the burning of fossil fuels. There is substantial scientific evidence that the accelerating global warming is a cause of greenhouse gas emissions. One of the main greenhouse gases is carbon dioxide [3]. Every year our earth′s atmosphere receives more than 15 billion tones of CO_2. Moreover, the combustion of fossil fuels is opined as a major contributor to the increase in the level of CO_2 in the atmosphere which is directly associated with global warming [4]. In this context the use of biofuels has the potential to greatly reduce our greenhouse gas emissions. However it is noteworthy that, biofuels generate about the same amount of carbon dioxide as fossil fuels, but every time a new plant grows, carbon dioxide is actually removed from the atmosphere. Therefore, the net emission of carbon dioxide will be zero as long as plants continue to be replenished for biomass energy purposes[5]. Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are composed of cellulose, hemicellulose, and lignin [6] and are considered as renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin [7].

A. Switchgrass

Switchgrass (*Panicum virgatum L.*) is one type of lignocellulosic feedstock that is suitable for cultivation on marginal land (with arid soil) without competing for cropland with other agriculture products [8]. Switchgrass is a hardy, deep-rooted, perennial rhizomatous grass that begins growth in late spring. It can grow up to 2.7 m high. The leaves are 30–90 cm long, with a prominent midrib. Its flowers have a well-developed panicle, often up to 60 cm long, and it bears a good crop of seeds. The seeds are 3–6 mm

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long and up to 1.5 mm wide, and are developed from a single-flowered spikelet. [9]. It is considered as one of the best second generation feedstock for bioethanol production due to the following economic, environmental and social benefits: (1) easy to grow, (2) low cost of production, (3) low soil nutrient requirement, (4) not consuming too much water, (5) high net energy yield per unit of cultivated land, (6) adapted to a wide range of environments including marginal soils and arid climates, (7) improved soil conservation, (8) reduction of greenhouse gas emissions, and (9) economic stimulation of underdeveloped rural areas [10,11]. Physical pretreatments such as mechanical grinding, pyrolysis, steam explosion, and ammonia fibre explosion (AFEX) can be effective in mechanical disruption of the cell wall and lignin bonds but have proven to be energy intensive. During ammonia fibre explosion pretreatment, only a small percentage of solid material is solubilized and no hemicellulose and lignin are removed [12,13]. The limitations of the steam explosion is that this method include the destruction of a part of the xylan fraction, incomplete disruption of the lignin–carbohydrate matrix, and generation of compounds that might be inhibitory to microorganisms used in downstream processes [14]. When carbon dioxide explosion was used to pretreat switchgrass, yields were relatively low compared to those of steam or AFEX pretreatments but high compared to that of enzymatic hydrolysis without pretreatment [15].

B. Ultrasound And The Sonication Process

Ultrasound is a mechanical acoustic wave with the frequency range from roughly 10 kHz to 20 MHz [16]. High-energy, or 'power ultrasound' has the frequency range of 16–100 kHz. Ultrasound ranging in frequency from 100 kHz to 1 MHz is regarded as 'high frequency ultrasound'. Low energy 'diagnostic ultrasound' has the frequency range of 1–10 MHz [17]. Only power ultrasound is sufficiently energetic to be used in biomass pretreatment processes, but low intensity sonication has the potential to improve the conversion of sugars to ethanol in the fermentation step. It imparts high energy to reaction medium by cavitation and secondary effects [18, 19]. In a typical dynamic process of cavitational bubbles, numerous microbubbles containing solvent vapors are generated that grow and undergo radial motion as acoustic energy propagates through the liquid medium. These microbubbles grow to a maximum of about 4-300 μm in diameter, and can be stable or transient. With low acoustic intensity, the radii of microbubbles periodically and repetitively expand and shrink (radial oscillation) within several acoustic cycles. While acoustic energy has sufficient intensity, some microbubbles are unstable within only one or two acoustic cycles. When the resonant frequency of bubbles exceeds that of ultrasonic field, the bubbles collapse within several nanoseconds, which creates special physical and chemical effects and enhances thermochemical/biochemical reactions or treatment [20]. The unsymmetrical collapse of bubbles at a broad solid/solvent interface (>200 mm) produces microjets at high speed (>100 m/s) toward solid surfaces. The instantaneous collapse of bubbles also produces strong shockwaves that might be up to 103 MPa [16]. This violent movement of fluid toward or away from the cavitational bubbles is defined as micro-convection, which intensifies the transport of fluids and solid particles and results in forces that can cause emulsification or dispersion depending on the conditions, while the strong shockwaves and microjets generate extremely strong shear forces over those of conventional mechanical methods and are able to scatter liquid into tiny droplets or crush solid particles into fine powders. [16]

The introduction of ultrasonic energy plays a positive influence on the pretreatment and thermochemical/biochemical conversion of biomass. Therefore, for the conversion of lignocellulosic biomass, the combination of ultrasonic energy and proper solvents allows the destruction of the recalcitrant lignocellulosic structure, facilitates the solvation and fractionation of biomass components and finally helps in increase of equilibrium yields of sugars, ethanol, bio-hydrogen and others products.

The present work reports the sono assisted pre-treatment of switchgrass followed by the analysis of the reducing sugars. Further, RSM will be used to optimise the sono assisted pretreatment conditions for high reducing sugar yields .

II. MATERIALS

Analytical grade liquid ammonia, glucose, sulphuric acid and sodium hydroxide purchased from spectrum® chemical mfg corp.. Sodium carbonate, sodium bicarbonate, calcium hydroxide dinitrosalicylic acid were purchased from Nice Chemicals Private Limited. All the chemicals were used as received from the supplier.

A. Switchgrass

Switchgrass was collected from NITK premises Surathkal, Mangalore, India. It was cleaned and dried in the oven. Further, the size of the dried switchgrass was reduced using a household mixer and screened to include particles with sizes lower than 2 mm and stored in polyethylene bags at room temperature.

B. Ultrasonic Equipment

The ultrasonic treatment was carried out using a probe-type sonicator (Sonics Vibra-cell VCX 130). The operating frequency and power of the sonicator were 24 kHz and 130 W, respectively. The amplitude was varied for RSM studies and the temperature was controlled using an ice bath. The ultrasonic processor probe was submerged in the center of the reactor.

C. Pretreatment

The Switchgrass powder was pretreated in 10mL vials. Initial studies were done keeping the solids ratio 0.2g/10ml of liquid medium, pretreatment time 10 min, amplitude 100%. RSM studies were done using different pretreatment conditions. Pretreated switchgrass was filtered using a vacuum pump and distilled water. It was dried in an oven for 24 hours and made to undergo acid hydrolysis. The different alkalis used for pretreatment were NaOH, $Ca(OH)_2$, Na_2CO_3, NH_4OH and $NHCO_3$. The alkali that gave maximum yield of % reducing sugar was used for the RSM studies.

D. Acid Hydrolysis

Pretreated biomass was taken in 10 mL vials and 5ml of 2wt% sulphuric acid was added to it. These vials were kept in a pressure cooker and made to undergo acid hydrolysis for 30, 60, 90, 120, 180 min. The hydrolysate was neutralised and pH was maintained between 7 to 8 and were tested for reducing sugars using DNS assay method. The time of acid hydrolysis that produced maximum yield of reducing sugars was used for further studies.

E. Sugar Analysis

Reducing sugars in the hydrolysates were determined by the dinitrosalicylic acid (DNS) method. In DNS assay, each sample of 0.3 was diluted in 0.7 ml distilled water and 1mL DNS reagent was added in glass tubes which were boiled at 100 ℃ for 5 min. The solution was made upto 10 ml and the absorbance was measured at 540nm. Glucose solution of 1 mg/mL was used for the calibration, hence the reducing sugar was measured as ‘‘equivalent glucose’’.

Total sugars were analysed using phenol-sulphuric acid method. Each sample was diluted 100 times using distilled water. 1mL of 5% phenol and 5 mL of conc. sulphuric acid was added to 1 ml of sample and kept in a water bath for 30-40min to cool. The absorbance of the sample was measured at 490nm.

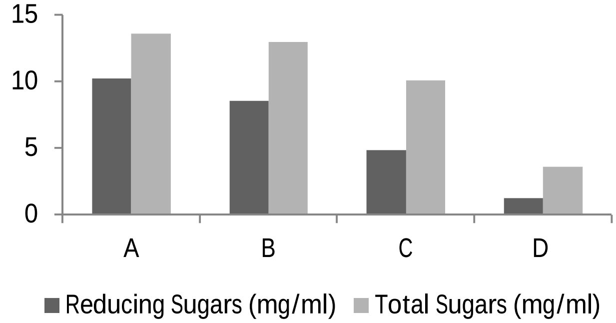
F. Experimental Design

Response surface methodology (RSM) was employed to obtain an optimal pretreatment condition that exhibits the highest reducing sugar yield (Y). To this end, four factors were used: Alkali concentration (A), Pretreatment time (B), Solids Ratio (C) and Amplitude (D). Tested conditions varied alkali concentrations of 0.0 wt.% - 5 wt% pretreatment time of 1- 10min, Solids ratio 0.1-0.2g/10 ml of liquid medium and amplitude 20%-100%. A total of 30 experimental runs with varied values of the four variables were carried out by Central Composite Design (CCD), and a center point was tested in triplicate. Data analysis was carried out via Design-Expert software (Version 7.0, Stat-Ease, Inc.,USA).

III. RESULTS AND DISCUSSION

A. Effect Of Sonication On Pretreatment Of Biomass

Switchgrass was made to undergo 4 different forms of pretreatments. Beaker 1 containing biomass was made to undergo sonication with sodium carbonate (alkali) as the liquid medium. Beaker 2 was made to undergo sonication with water as the liquid medium Beaker 3 and beaker 4 were filled with sodium carbonate and water respectively and were not subjected to sono assisted pretreatment. A 10% improvement in the yield of reducing sugars could be obtained in case of biomass pretreated with



Na_2CO_3 than with water which can be inferred from Fig 1. Fig. 1 Effect of sonication on pretreatment of biomass using , A-biomass+sodium carbonate+sonication, B-biomass+water+sonication, C-sodium carbonate (no sonication), D- water (no sonication).

Further, it was also noted that the involvement of sonication and Na_2CO_3 in the pretreatment of the biomass resulted in better yields of reducing and total sugars in comparison to that of the untreated biomass. The %reducing sugar content in biomass which was pretreated along with Na_2CO_3 was 75.31% reducing sugars out of the total sugars whereas in biomass which was pretreated using water had 65.79% reducing sugars. This improvement may be attributed to the hydrodynamic shear forces associated with sonication. Also, the alkaline pretreatment is known to cause solvation and saphonication of biomass that creates swelling. This biomass swelling enlarges the internal surface area of cellulose and decreases the degree of polymerization and crystallinity.

B. Effect Of Time Of Acid Hydrolysis

It was observed that the maximum yield of reducing and total sugars were found after the biomass was hydrolysed upto 90 min as seen in figure 2. Almost 2.6 mg/mL of reducing sugars per 0.2 g of biomass was obtained. Therefore 90 min was considered as the optimum time for acid hydrolysis. A partial hydrolysis of the biomass followed by complete degradation of sugars was observed at 60 and 120 min respectively (Fig 2). The decrease in amount of total and reducing sugars at higher times of acid hydrolysis is caused due to sugar degradation. This degradation normally occurs in acid hydrolysis and its velocity depends on temperature and acid concentration.

C. Effect Of Liquid Medium On Pretreatment Of Biomass

It was observed that among all the three alkalies that had been used for the pretreatment, 2wt% Na_2CO_3 gave the highest yield of 64.44% reducing sugars which was 20% higher when compared to 2wt% $CaOH_2$ which was 2 wt% 44.28% and NaOH which was 26.06% reducing sugars . From fig. 3 we can see that the highest yield was observed

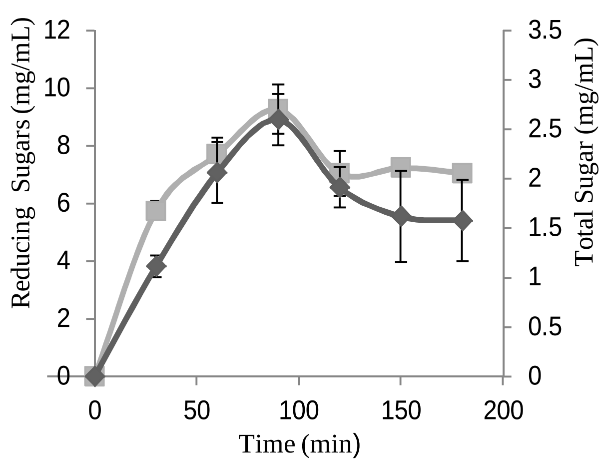


Fig.2 Effect of time of acid hydrolysis on reducing sugars and total sugar yield.

when a weak base was used. Weak bases were explored in the future study.

2wt% NH_4OH, 2wt% Na_2CO_3 and 2wt% $NaHCO_3$ were the other weak bases that were used for the pretreatment of switchgrass. It was seen that 2wt% NH_4OH gave the highest percentage of reducing sugars, which is 52.63% in comparison to 2wt% Na_2CO_3 and 2wt% $NaHCO_3$ which showed 46.1% and 31.87% of % reducing sugar respectively. To confirm that NH_4OH gave the maximum yield, different concentrations of all the 3 weak bases were taken and the reducing sugars were analysed. From fig. 5 we can infer that NH_4OH gave the highest yield of reducing sugars. This might be attributed to the combined effect of ammonia and hydroxyl radicals formed during the cavitation which enhanced the depolymerization of lignin and cleavage of lignin-hemicellulose linkages. One of the promising pretreatment methods is ammonia pretreatment in a flow-through column reactor. According to Kim et al [20], this method was highly effective in delignifying biomass, reducing lignin content by 70-85%. Ammonia was chosen as a pretreatment reagent because it is widely available and residual ammonia will serve as a nitrogen source for fermentative organisms.

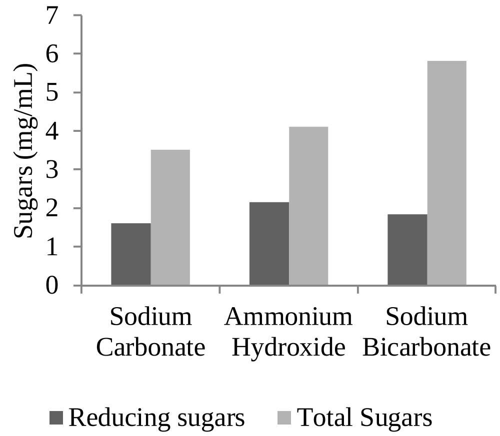


Fig. 4 Effect of weak base on reducing sugars and total sugars yield.

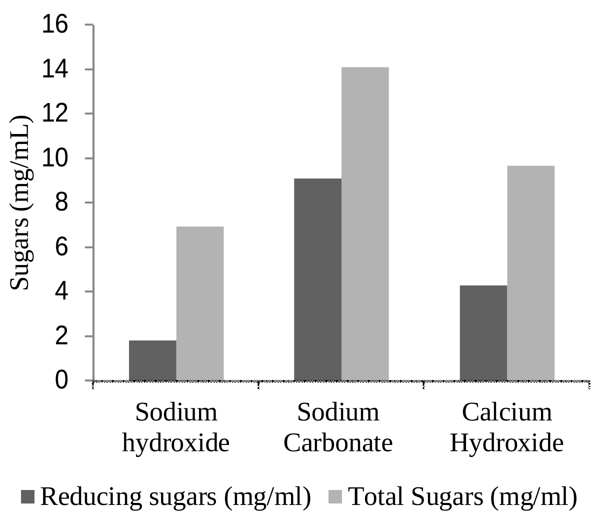


Fig. 3 Effect of Alkali on reducing sugars and total sugars yield

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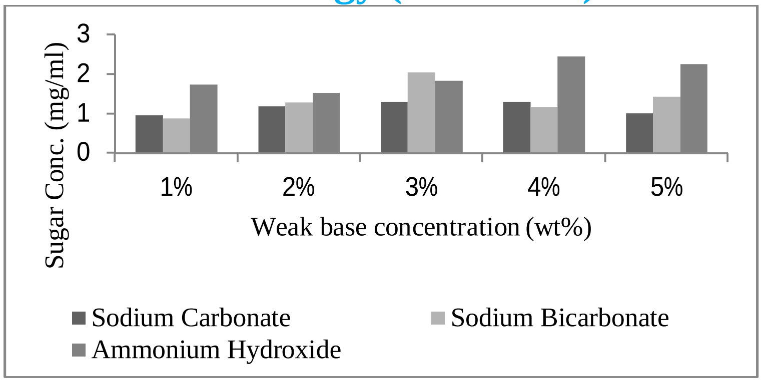

Figure 5. Effect of range of concentration of weak base on reducing sugar yield

D. Optimization Using Response Surface Methodology

Central composite design

The CCD model offers useful data on direct, pairwise interaction, and curvilinear variable effects. The influence NH_4OH concentration (A), sonication time (B), Solids Ratio (C) and Amplitude (D) on reducing sugars response is shown in table 1.

The following empirical regression equation represents the Reducing sugars content as a function of ultrasonic intensity NH_4OH concentration(A), sonication time(B), Solids Ratio (C) and Amplitude (D).

Table 1 ANOVA table for reducing sugars

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Block	3.19	4	0.80			
Model	9.61	14	0.69	16.67	< 0.0001	significant
A-concentration	1.263E-003	1	1.263E-003	0.031	0.8642	
B-time	0.023	1	0.023	0.57	0.4661	
C-solids ratio	7.47	1	7.47	181.50	< 0.0001	
D-amplitude	0.54	1	0.54	13.07	0.0041	
AB	9.082E-003	1	9.082E-003	0.22	0.6478	
AC	0.025	1	0.025	0.60	0.4536	
AD	4.206E-003	1	4.206E-003	0.10	0.7553	
BC	0.33	1	0.33	8.10	0.0159	
BD	7.840E-006	1	7.840E-006	1.904E-004	0.9892	
CD	0.17	1	0.17	4.20	0.0649	
A^2	0.19	1	0.19	4.54	0.0564	
B^2	0.093	1	0.093	2.25	0.1614	
C^2	0.68	1	0.68	16.40	0.0019	
D^2	0.32	1	0.32	7.74	0.0178	
Residual	0.45	11	0.041			
Lack of Fit	0.42	7	0.060	7.17	0.0378	significant
Pure Error	0.033	4	8.356E-003			
Cor Total	13.26	29				

The model F-value of 16.67 with a low p-value ($p < 0.0001$) implies that this model was statistically significant at 95% confidence level. In addition, the model terms C, D, BC, C^2, D^2 which are time ,amplitude, there squared terms and the interaction effect of time and solids ratio had significant effect on the reducing sugar yield, since each p-value is less than 0.05. The experimental values showed high correlation (R^2 = 0.9550) with predicted values.

Fig. 6 Shows a rounded ridge running diagonally on the plot, indicating that time of pretreatment and concentration of liquid medium were slightly interdependent, or that they had significantly interactive effects on reducing sugar yields. The optimised conditions obtained for the maximum yield of reducing sugar were NH_4OH concentration 2.5% pretreatment time 5.5min with the solids ratio of 0.15g/10mL of liquid medium and sonication amplitude of 55%. These conditions were used and an experimental run was carried out and reducing sugars obtained were 2.679mg/ml = 66.975 mg of reducing sugars / gram of biomass. This tallied with the obtained point prediction. This indicates that the pretreatment conditions for maximum reducing sugar yield from switchgrass (*Panicum virgatum L.*) were successfully optimized via central composite design.

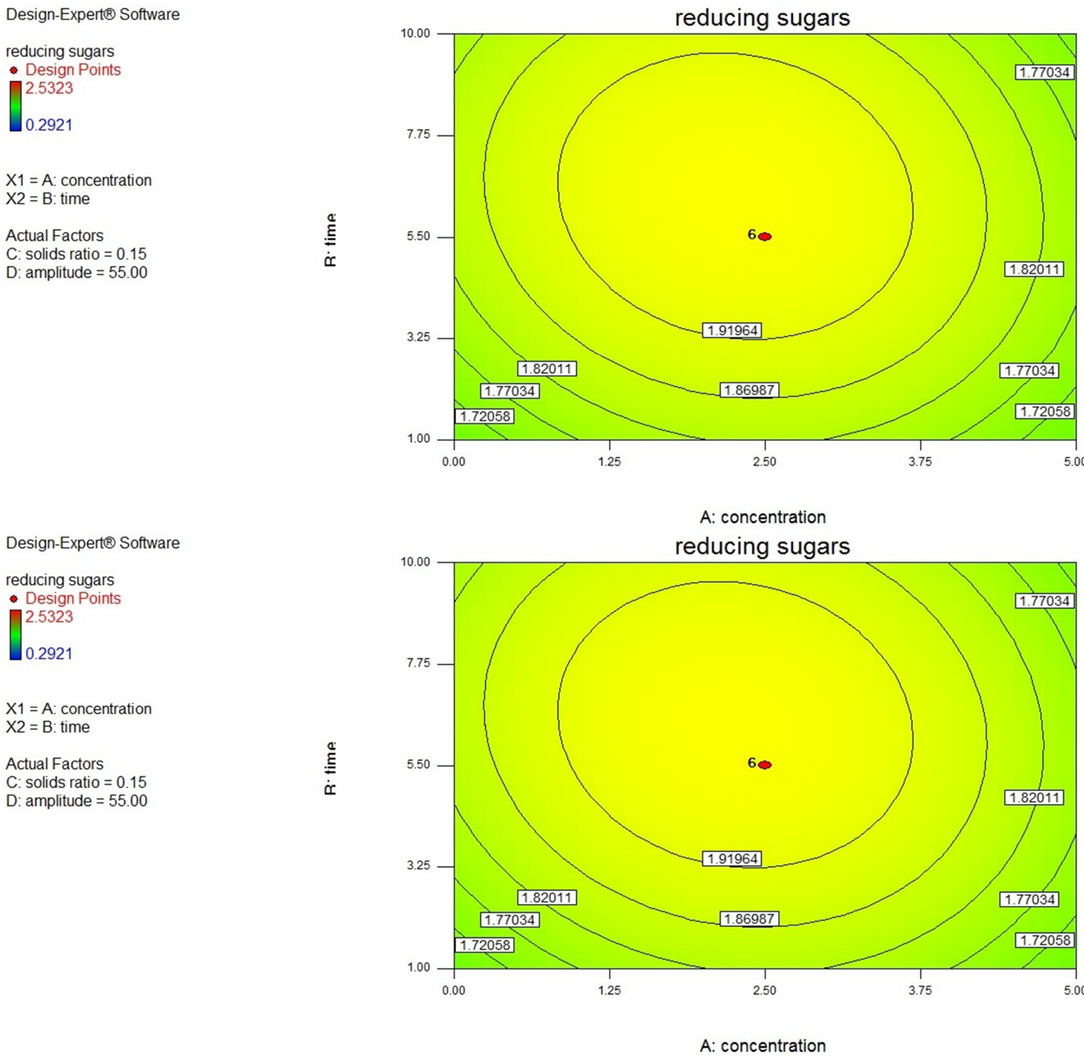

Fig. 6 Contour plot of reducing sugars and amplitude as a function of time and concentration.

IV. CONCLUSION

In this study, ultrasonic technology was used to pretreat switchgrass and analysis of reducing sugars were done. Better yields of sugars were observed when the effects of sonication and alkali were involved. Weak bases are observed to be better liquid mediums that can be used for Sono assisted pretreatment. The acid hydrolysis time that gave the maximum yield of sugars was at 90min. Out of all the weak bases NH_4OH gave the maximum yield of reducing and total sugars.

The optimum conditions obtained after response Surface Methodology studies were Ammonia concentration 2.5 wt%, time of pretreatment 5.5min, solids ratio 0.15g/10mL of liquid medium, amplitude 55%. The reducing sugars that are obtained after these conditions were used were 2.679 mg/mL of reducing sugars per 0.2 g of biomass. = 66.975 mg of reducing sugars / gram of biomass.

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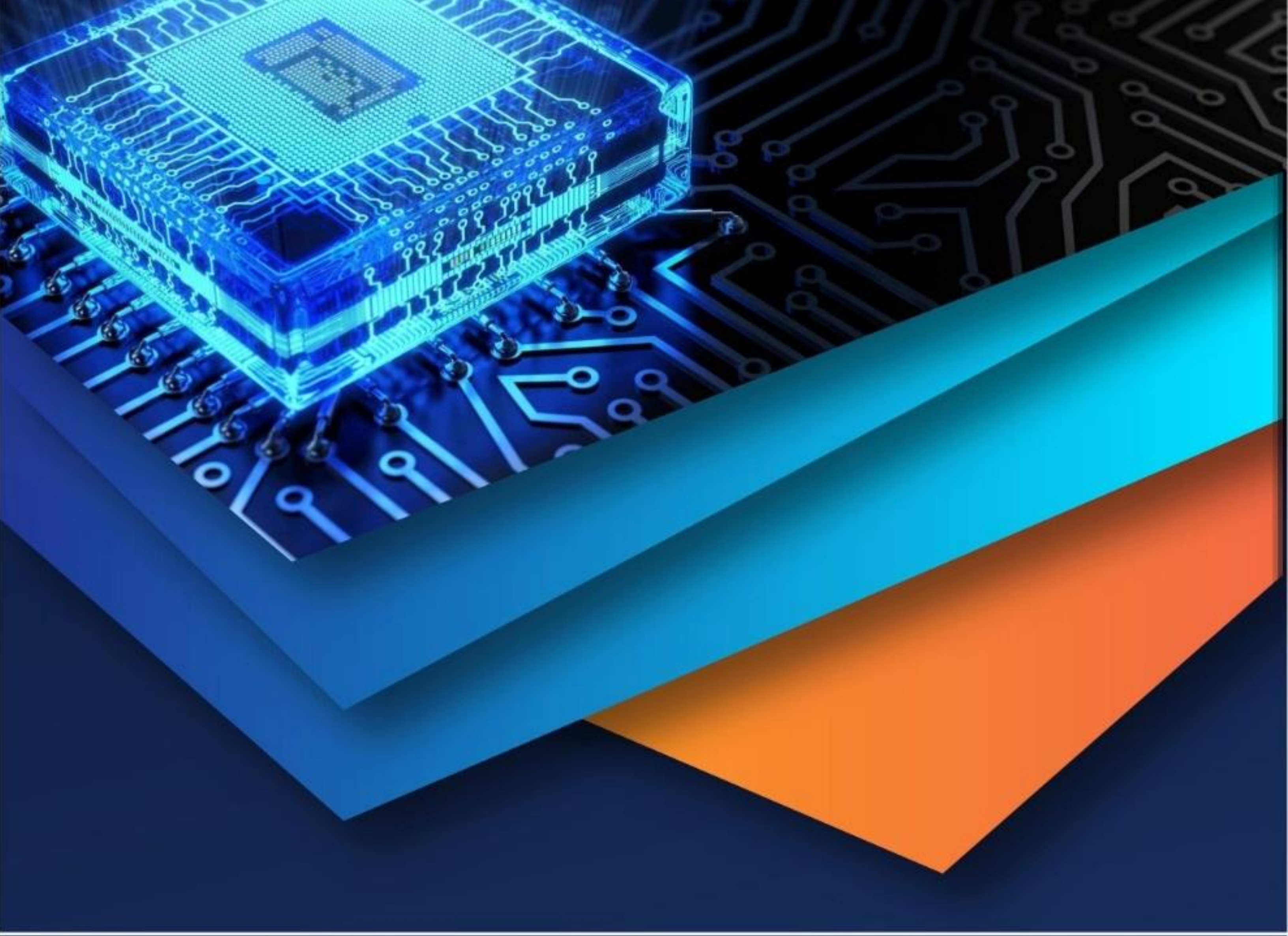

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